SOP 1: Sampling Site Selection, Establishment and Description

These instructions guide selection, establishment, description, and rapid assessment (**RAD**) of sampling sites used for monitoring monarch butterfly populations and their habitat. Establishment of sampling **plot**s at designated locations includes checking the agreement of mapped characteristics in a potential plot area with the original intended land-use/cover category (**sample draw stratum;** Table SOP-1.1) identified for being sampled. Description of a selected site entails verifying a vegetation type (National Vegetation Classification or NVC) following O'Brien and Knutson (2015) at plot center and recording any disturbances that have occurred with more than 10% of the sampling plot area.

Selection of suitable plots proceeds more quickly with initial mapping and screening of potential sampling locations designated by a sample draw Supplemental Materials (SM) 1. Inspection within a Geographic Information System (GIS**)** and calculation of the amount of sample draw stratum area within each plot provides advance screening for acceptance or rejection of a plot, and saves time on the number of sites that have to be visited before establishing sampling plots.

For this trial, sites that qualify for plot establishment and rapid assessment should have at least 75% of the plot area classified as the same sample draw stratum indicated by the **sample draw location** (or Generalized Random Tessellation Stratified [GRTS] point), but with a preference to encompass 100% of its original stratum (SM 1). When two or more possible plot layouts for the same sample draw positions include the same and great amount of area in the sample draw stratum, then the plot area with the greatest range (lowest to highest) in elevation (m), should be chosen for sampling. This latter criterion provides greater opportunity for including more species of nectar plants in a plot. Portions of any plot that are found to be of a different stratum will not be subsampled and measured.

Describing the site during the first visit will include verifying that the expected sample draw stratum and mapped vegetation type are correct. Data collectors will need to know the names of and characteristics of expected and other local strata or vegetation-type categories before visiting the site. Data sheets can be pre-populated with the expected stratum name and vegetation-type name. One advantage of this approach is that field staff with minimal botanical training can verify that the associated vegetation type is found at the sampling location, or, if the assignment is incorrect, can assign another vegetation type from a short list of those found in the study area.

Attributes Measured or Assessed

- Percent homogeneity of plot area (non-roadside plots) with original sample draw stratum
- Range of elevation (m) in a plot
- Vegetation type (NVC Macro and Ecological System names)
- Land use and disturbance (categories)
- Time (hrs) required to conduct this SOP
- Relative amount of milkweed (*Asclepias* spp.) plants
- Relative amount of herbaceous plants

Equipment and Materials

- GPS enabled Personal Accessory Device (PAD or iPAD) with coordinates for sampling site locations and plot boundaries or paper map (recommended scale: 1:24,000; with topography or aerial photo background and UTM coordinate grid)
- Documentation showing NVC classes and descriptions at sampling locations (with location points identified by sampling design strata, pre-screening results, and priority of selection)
- Compass with sighting mirror and declination scale
- Data sheets, clipboard, pen/pencil (or PAD and stylus)
- 100-meter tape
- Wire flags (glow-pink, white, orange) and black marker
- Camera (stand-alone, PAD or phone)

Data Entry

Record information on (see also SOP 5, SMs 4 and 5):

- Conditions (including disturbances) and location of each sampling site and plot using iPad survey form named SOP1_SiteDescription or paper form labeled SOP 1 sheet 1;
- Rapid assessment of milkweed plants and plant community composition using iPad survey form named SOP1_RapidAssessment or paper data form labeled SOP 1 sheet 2;
- Miscellaneous observations of adult monarch butterflies by distance and behavior (iPad survey form named SOP2_AdultMonarchObservations or use paper data forms labeled SOP 2 sheet 3).

How Are Vegetation Classes Standardized and Mapped?

Standardized, mapped vegetation types are readily identifiable by trained observers in the field and represent "ecological systems", or recurring groups of biological communities (Comer et al. 2003). Several federal and NGO agencies employ these standards and have developed useful tools; we will employ the USGS Gap Analysis Program (GAP). The GAP maps use vegetation classes from NatureServe's Ecological System Classification (Comer et al. 2003) and the National Vegetation Classification System. These are the same vegetation classes used by the LANDFIRE program to model fire behavior and predict disturbance potential. The GAP map covers the entire U.S. including Alaska, Hawaii, and Puerto Rico.

The standardized vegetation classifications (defined hierarchically as Class, Formation, Macrogroup, and Ecological System) for a state, county, or Landscape Conservation Cooperative geography can be perused with the GAP Land Cover Data Viewer. If you click a location on the map, a description of the class and a range map pop up. This tool can be used to generate a master list of the vegetation classes in the vicinity of the study area. Full descriptions of the classes are available from NatureServe Explorer for states, provinces, Forest Service Ecoregions, and MRLC 2000 Map Zones. For example, a search for 'oak', with Wisconsin selected as a state, turns up a list of classes, one of which is 'North-Central Interior Dry Oak Forest and Woodland'. A detailed description is provided.

The survey coordinator will need to prepare either a PAD or paper field maps of pre-screened locations for conducting rapid assessments and data sheets that give the expected stratum (Table

SOP-1.1) and NVC Macrogroup and Ecological System (Table SOP-1.2). See SM 1 for additional details.

Table SOP-1.1. Description of sampling strata in which sample units (sites) were allocated in a Generalized Random Tessellation Stratified (GRTS) sample draw and then partitioned further into a sampling strata.

Sample Draw Stratum	Sampling Stratum	Code	Identifying Features
Grassland	Protected Grassland	PRG	Herbaceous plants dominant; public/protected land
Grassland	Unprotected Grassland	UPG	Herbaceous plants dominant; private/unprotected land
Agriculture	Agriculture Crops	AGC	Primary crops (corn; soybean), other crops, orchards
Agriculture	Agriculture CRP	CRP	Fallow fields to restored grassland; land enrolled in the Conservation Reserve Program
Rights-of-way	Road side	RDS	Roads with speed limits < 55 mph

Pre-screening Plots

1. Procure the coordinates, draw priority, and descriptive data for the list of plots that need to be rapidly assessed and described (see example information in Supplemental Materials (SM) 1.
2. Identify the **plot label**. Every data sheet needs to show this label because it will include the **sampling stratum** name and is what will link the data collected to the location on the ground. The plot label is coded according to the sampling strata code (Table SOP-1.1), and a number indicating the order of priority for sampling a site within that stratum (e.g., first roadside plot to be examined for sampling will be labeled RDS01). Plot labels are only unique to a **monitoring area.**
    a. In 2016, there are three National Wildlife Refuges (Neal Smith, Waubay, and Washita) and four urban-suburban areas near Chicago, IL, Kansas City, KA, Minneapolis, MN and Austin, TX that are identified as monitoring areas.
    b. For example, of the 3 areas that included refuges could have plots labeled RDS01.
3. Examine the area within a plot against an aerial photo background (like Google Earth), and relative to refuge boundaries, to detect any conditions for dropping the plot from sampling (e.g., plot straddles refuge boundary and includes PRG and UPG sampling strata; more than 25% of a plot area has non-monarch habitat like open water or dense forest).
4. Pre-screen roadside plots in a GIS or on aerial photograph in advance to determine direction to proceed with set up and whether a road meets the minimum requirements. Use the process detailed in SM 1 to identify coordinates for start and end-points of each transect (8 on either side of the road). Pre-screening is particularly important for saving time for determining how to place transects in around curving roads.
5. For off refuge sites that are chosen for sampling independent of the initial sample (GRTS) draw, pre-screening will be needed to determine coordinates for plot corners or transect start and end locations. Naming of these plots will similarly include the three-

character acronym (Table SOP-1.1) but the two digit number will need to be 90—99 (e.g., UPG91 for a unprotected grassland selected for sampling on a private land neighboring the refuge but not allocated in the GRTS draw for the 60-mile radius general sampling area around the refuge).

Field Methods

Locate sites and establish plots—After initial screening, complete the following steps to locate and set up plot boundaries:

1. Locate the priority draw sites and find the NW corner (grassland and agricultural plots) or a starting point (road side plot) from the UTM coordinates in the iPad or paper map (Figures SOP-1.1 and 1.2).
2. Record the start time (24-hr clock) for implementing this SOP, once moving with your gear from the vehicle to find the starting point for marking a plot boundary.
3. Set in the boundaries or starting marks of the plots according to the type of stratum being sampled (Figure SOP-1.1). Use a compass (with set declination for your area) and a meter tape for distances from an identified corner. See Figure SOP-1.1 for distances and directions among markers. For all UPG, PRG, and CRP plots, directions will always be north (0º), south (180º), east (90º) or west (270º). Directions for RDS and AGC plots will vary and depend on the direction of the road or plowed furrows, respectively (Table SOP-1.2). Go to the northern most corner of the field that is formed by the edge that runs parallel to the crop rows and the edge that is at the start of the rows (i.e., you can peer down the furrows between the crops).
    a. Mark corners and mid-points on the long sides of the **PRG, UPG and CRP plots** with glow-pink or and bamboo stakes (6 flags and 6 bamboo staffs per plot). Mark intervening 75-m segments on the perimeter with orange flags (4 total; Figures SOP-1.1a and b). Record or upload the coordinates if not already provided by the iPad or GPS.
    b. Place white flags for plant SOP 3 plant subsampling after it is known that a plot will be sampled more intensively than for the rapid assessment.
    c. At **RDS plots**, use the coordinates developed during pre-screening to locate and mark the start and end points for each of 8 transects on both sides of the road with white wire flags (total of 32 flags). The end point of Transect 8 should occur at the **road width** distance measured perpendicular (90 degrees) to the direction of the road, in line with the sample draw (GRTS) point and extended to either:
        i. edge of the first obvious change in vegetation type, or
        ii. a maximum of 30 meters, and
        iii. a minimum distance of 6 meters.

        The starting point for Transect 9 should be marked on the same perpendicular direction from the sample-draw point at the edge of the road. In this manner the full dimensions of the roadside plot can be identified from the end points of Transects 8 and 16 and the beginning points of Transects 1 and 9 (Figure SOP-1.2). Record or upload coordinates for the 32 points marking the start/end of the 16 transects through the iPAD (SM 4) or with a separate GPS.
    d. At **AGC plots**, when permission is granted by the land owner or manager, enter and mark the four corners of the extent of sampling in agricultural fields with crops similarly to a grassland plot. However, unlike a grassland plot, the orientation of the

AGC plot is not strictly in the 4 cardinal directions. The orientation of AGC plots will determined by the tillage pattern of crop rows and plot dimensions will be variable depending on the number of 75-m transects that can be placed in a single row (Figure SOP-1.1c).

e. To establish the plot boundaries identify the northwest-most corner of the agricultural field (Figures SOP-1.1c and d). From this corner, count the randomly selected numbers of crop rows (Table SOP-1.3) to find the first axis of the plot that is perpendicular to the crop rows (Figure SOP-1.1c). The first series of transects will be placed on the northern most side of a randomly selected crop row. If not calculated during pre-screening, determine where the last transects and second perpendicular axis for the plot will occur. Finish marking the plot corners and record or upload the coordinates of the marks using the iPad or GPS.
f. When traveling to the next set of transects move to a different furrow that is adjacent to 1 or 2 more crop rows and walk to the closest edge of the agricultural field. Measure out to the number of rows needed to get the second perimeter length of the agricultural field. Use Table SOP-1.3 to identify this part of the plot boundary that will be the location for the last set of transects in the field.
g. Unless instructed otherwise, leave the flags in place for future data-collection visits. Once a site will no-longer be sampled, retrieve any flags or bamboo poles. You will likely need to remove any flags from AGC and RDS plots following each visit after the rapid assessment.

Additional marking will be made on the plots, once the data from the rapid assessment diagnostic (RAD) of several sites has been examined and the plots needed for intensive sampling have been identified (see *Marking transects* below).

Table SOP-1.2. Quadrants and compass bearings used for assigning direction to set up roadside (RDS) plots.

Quadrant for anchor point	Anchor point degree range	Road direction bearing range	Quadrant for road direction	Travel vector
East to South	90—179°	270—359°	West to North	
South to West	180—269°	0—89°	North to East	

Example: The first AGC plot needs to be established in a 300 m x 600 m (18,000 m^2 or ~4.5 ac) sized field of wheat. The edge of the field that runs parallel to crop rows is 300 m long. Spacing among the wheat rows is 10 inches or 20 rows per 75 m. Where are the 4 corners on the AGC plot placed?

Answer: Pre-screening shows that the number of rows needed to house all 16 transects used in SOP 3 within a field that has an edge length of 300 m and 0.25 m between rows will require 100 rows from randomly selected starting row because each row can only hold 3 transects (4 transects, plus 5 m between would require, 320 m) and there are 20 rows per 75-m distance, which is the required spacing among sets of transects in an AGC plot. This field needs is 600 m

long on the side perpendicular to the crop rows and should have 600 m/0.25 m between rows = 2,400 rows, meaning that the 16 transects will easily fit in this AGC plot.

Counting from the NW-most corner of the field down to the 6th row, the NW-most corner flag for this plot would be placed 5 m from the edge in the furrow on the north side of row 6. This is the beginning of the first 75-m transect. The NE-most corner of the plot would be at the end of the 3rd transect or 240 m from the edge of the filed in row 6. The last (16th transect) is 100 rows from the first flagged row. This means the SW-most corner is placed at the start of the 16th transect, 5 m from the edge of the field and the north side of row 106. The-SE most corner coordinates would be marked by moving 160 m along the north side of row 106 from the end of Transect 16 (Figure SOP-1.1d).

Table SOP-1.3. List of randomly selected number of crop rows for identifying northern-most boundary of agricultural (AGC) plots, and the number of crop rows needed to house all transects within a plot for fields of different dimensions and type of crop. Example use is given below the table.

Locate First Row	Determine No. of Transects per Row		Determine Location of Different Sets of Transects			
Start Row	Length (m) of 1 Row	Transects (75 m + 5 m) per Crop Row	Typical Crop	inches	meters	No. rows in 75 m
6	<80	0	Wheat or Barley	7.5	0.19	15
9	80	1	Wheat or Barley	10	0.25	20
1	160	2	Wheat or Barley	12	0.30	23
10	240	3	Corn or Soybean	15	0.38	29
4	320	4	Corn or Soybean	20	0.51	39
3	400	5	Corn or Soybean	30	0.76	58
7						
8						
2						
5						

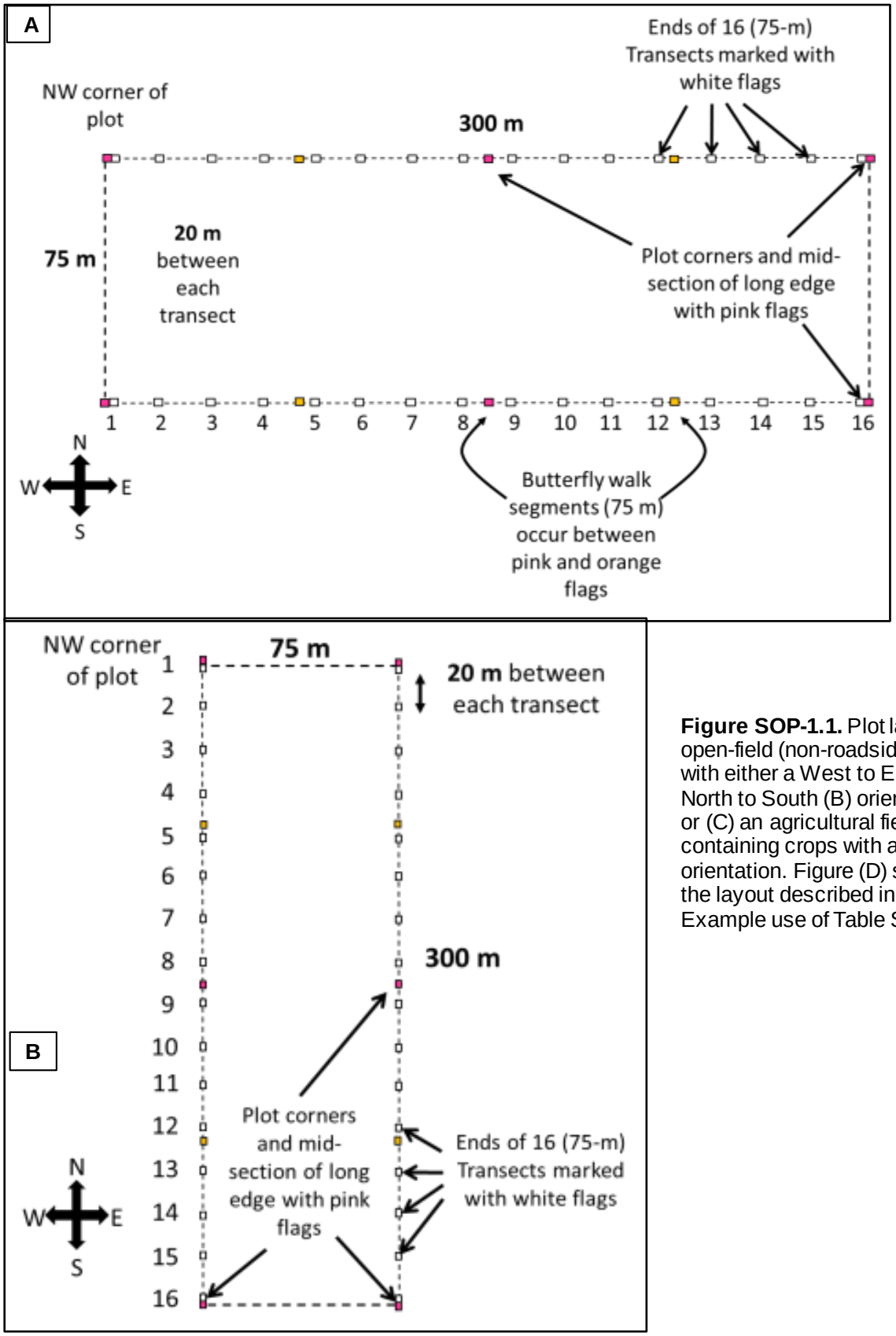
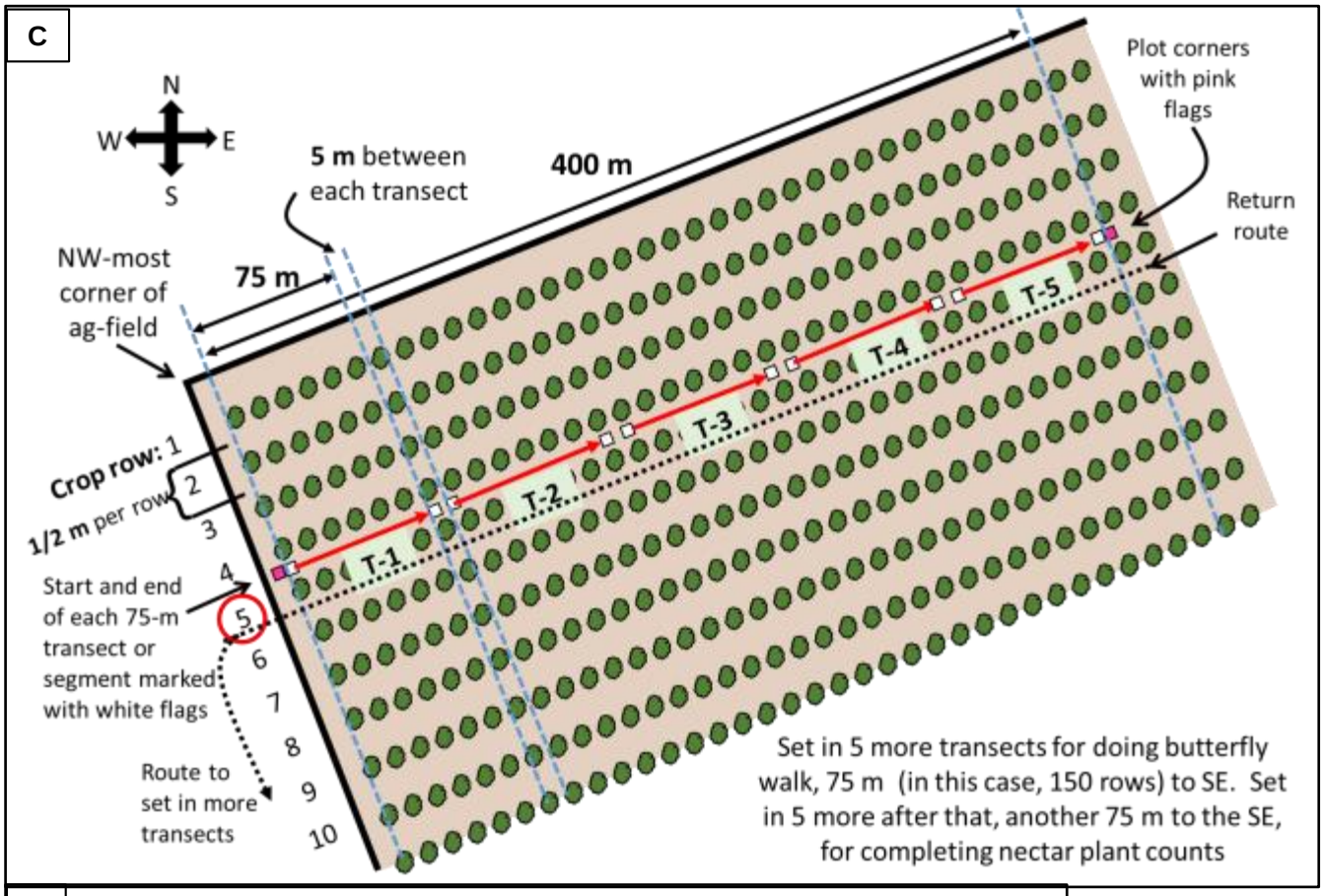
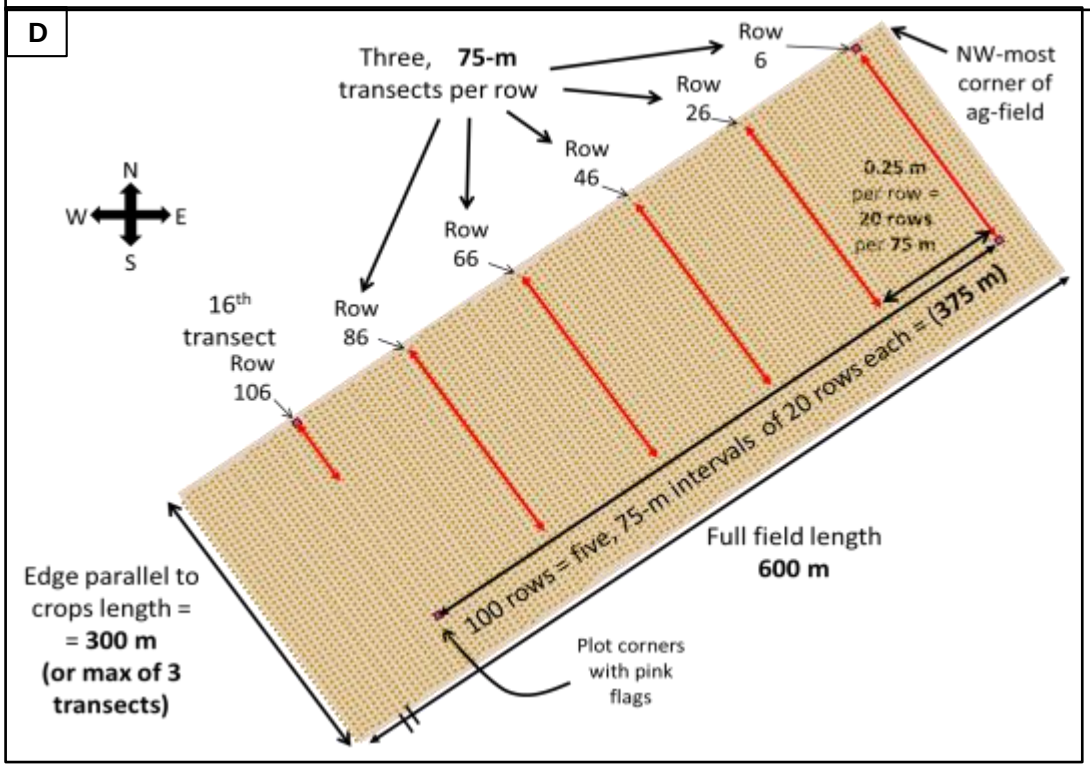


Figure SOP-1.1. Plot layout for open-field (non-roadside) plots with either a West to East (A) or North to South (B) orientation, or (C) an agricultural field containing crops with any orientation. Figure (D) shows the layout described in the Example use of Table SOP-1.3.


C
N
W
E
S
5 m between each transect
400 m
75 m
Plot corners with pink flags
Return route
NW-most corner of ag-field
Crop row: 1
1/2 m per row
2
3
4
5
6
7
8
9
10
T-1
T-2
T-3
T-4
T-5
Start and end of each 75-m transect or segment marked with white flags
Route to set in more transects
Set in 5 more transects for doing butterfly walk, 75 m (in this case, 150 rows) to SE. Set in 5 more after that, another 75 m to the SE, for completing nectar plant counts


D
Three, 75-m transects per row
Row 6
Row 26
Row 46
Row 66
Row 86
16th transect Row 106
NW-most corner of ag-field
N
W
E
S
0.25 m per row = 20 rows per 75 m
100 rows = five, 75-m intervals of 20 rows each = (375 m)
Full field length 600 m
Edge parallel to crops length = = 300 m (or max of 3 transects)
Plot corners with pink flags

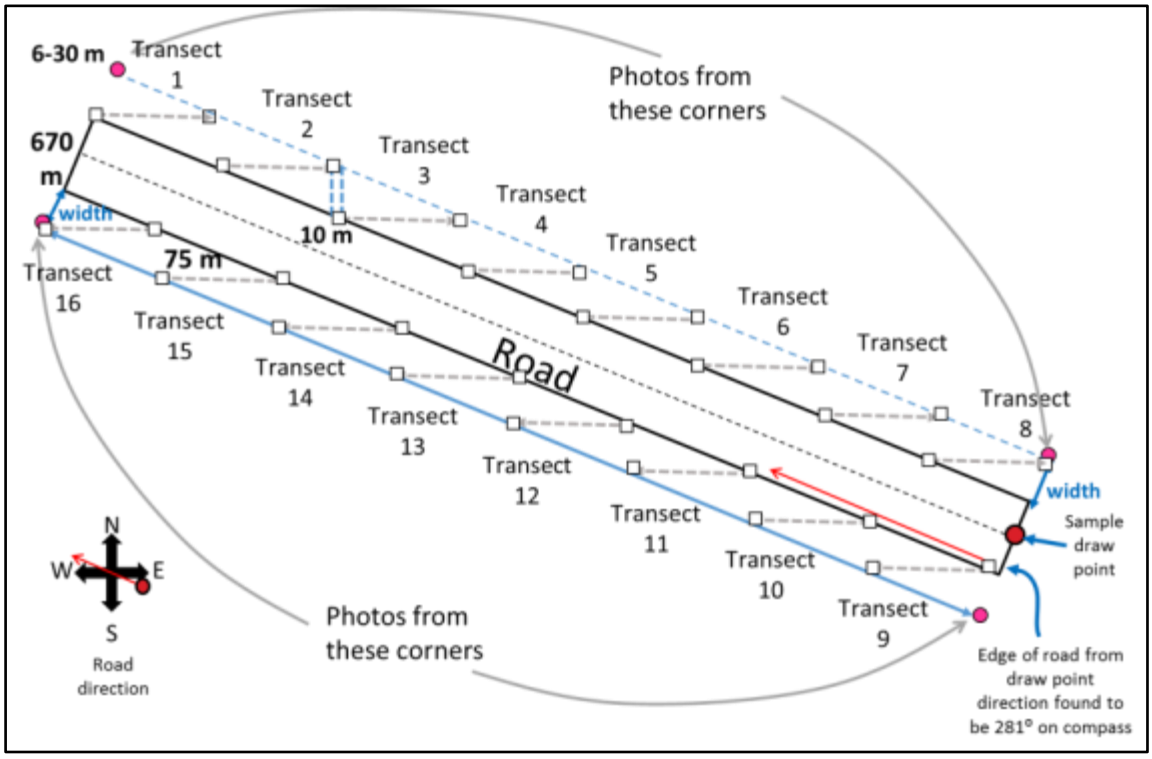


Figure SOP-1.2. Plot layout for roadside plot comprised by 8 diagonal transects (each 75 m long) on both sides of a road. Each transect is separated by 10 m and starts at the edge of the road and ends at the roadside width defined by edge of the roadside vegetation type with an adjacent different type (or 30 m, whichever occurs first). Minimum width is 6 m.

Describe the Plot and Conduct a Rapid Assessment (RAD)

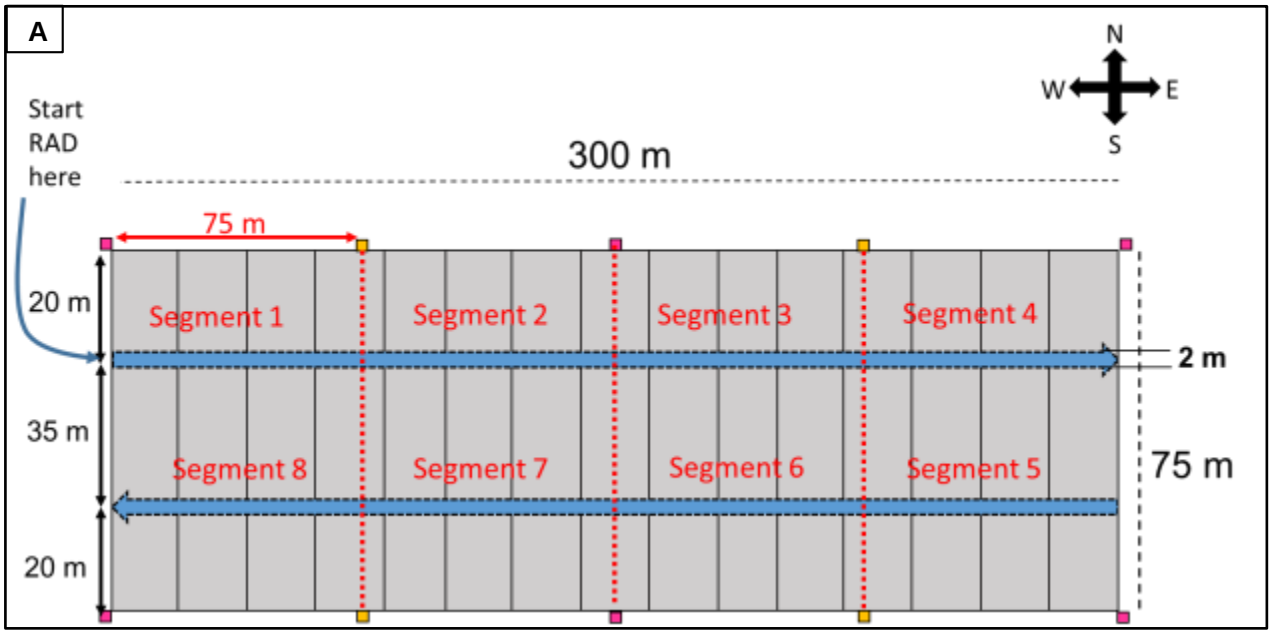
After or while marking out a plot, describe the plot and conduct a rapid assessment diagnostic (RAD). During the RAD you will record milkweed abundance by species and general vegetative community in the plot area. This only has to be recorded during the first visit to a site.

1. Take the proper photographs of the plot interior from the corners.
    a. Take photographs from the four corners facing inward toward the plot center at a 45 degree angle. If not recorded in the photo, make sure to record the information shown in Table SOP-1.4 on Data Sheet 1 for SOP 1.
    b. At roadside plots, take photos from the beginning and end marks of the series of 8 plots at the road's edge, also facing 45 degrees towards the center of area covered by the 8 plots on both sides of the road (Figure SOP-1.2).
2. Return to the NW corner (non-roadside plots) or start mark for Segment 1 and conduct a RAD and verify the NVC when closest to the center of the plot.
    a. Walk from the corner along the short edge of the grassland plots to the starting point of the RAD Segments (Figures SOP-1.3a and b).
    b. Walk the RAD transects and look in a 2-m belt around each transect (1 m on each side), and tally the number of milkweed stems seen by species. Record the tallies for each 75-m segment (8 total on SOP 1 Data Sheet 2).

c. For the roadside plot RAD, walk the 8, odd numbered transects (Figure SOP-1.3c). Record your tally of **milkweed plants/ramets** (or stems, see the SOP 3 and Glossary) by species, for each transect.
d. If conducting a RAD in the AGC plot, conduct within the rows containing the first 4 transects and the last 4 transects (for 8 Segments total).
e. Fill out Plant Community Checklist (backside of SOP 1 Data Sheet 2).
f. When near the 150-m mid-point of the long side of the grassland plots, determine if the expected NVC Macrogroup and primary Ecological Systems are correct for classifying the center point of the plot. If not, indicate the correct classification names indicated by the NatureServe Explorer descriptions of the NVC categories for your area on SOP 1 Data Sheet 1.
g. Verify the Ecological System for a RDS plot at the roadside looking away from the road, ½ the road-width distance in a perpendicular direction from start of Transect 5 and again from start of Transect 13.
h. Indicate if a secondary Ecological System is observed within 100 m of the plot boundary and give the system name.

Table SOP-1.4. Core information (metadata) that should be recorded for each photo taken at the corners of each plot.

Photo Metadata
Photo Identifier (image no. of file name)
Location Data (UTM x-y coordinates; zone)
Monitoring Area and Plot Label
Corner Identifier (NW, NE, SE, or SW)
Photographer's Name
Calendar Date (mm/dd/year)



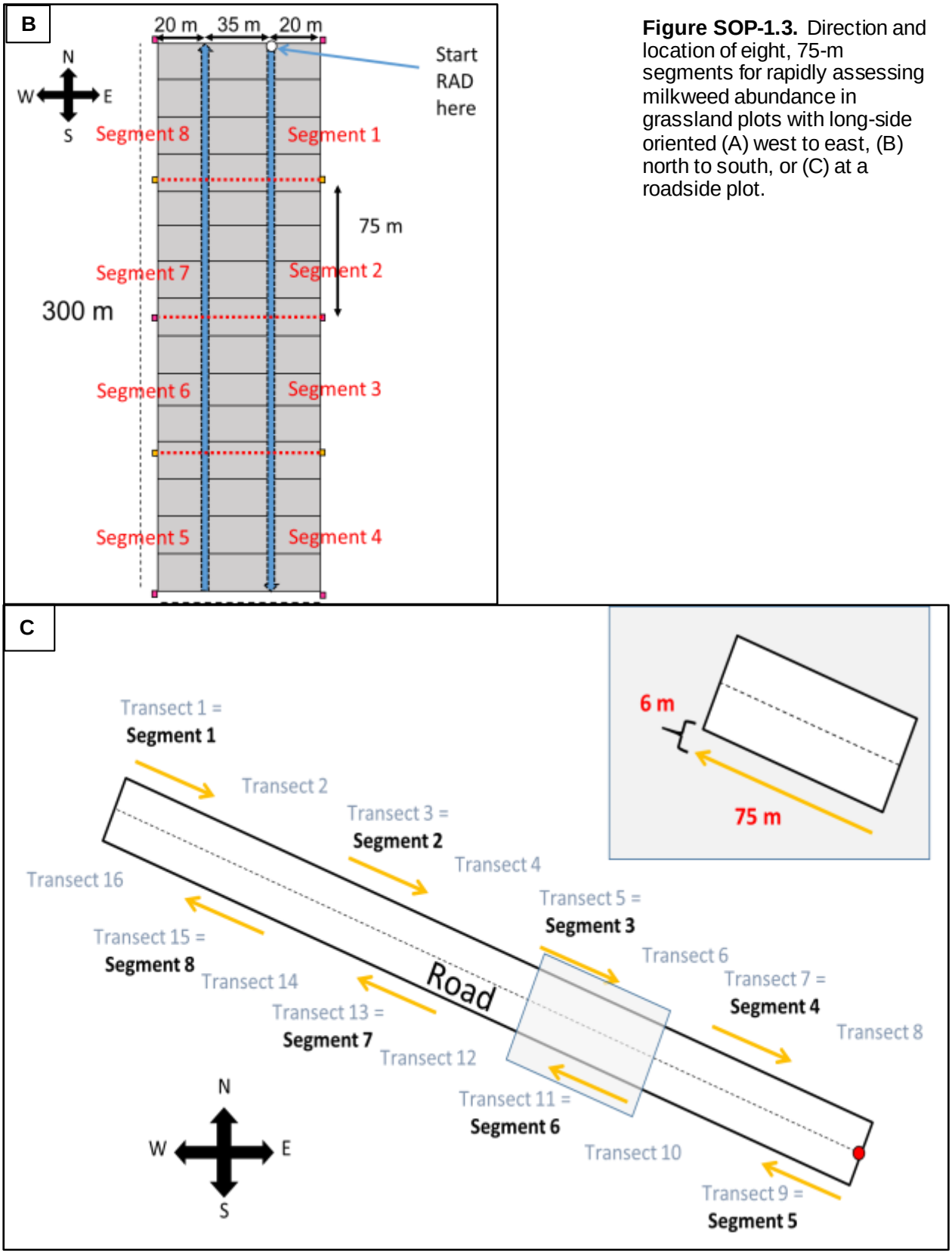


Figure SOP-1.3. Direction and location of eight, 75-m segments for rapidly assessing milkweed abundance in grassland plots with long-side oriented (A) west to east, (B) north to south, or (C) at a roadside plot.

Record Disturbance—Natural and human-induced disturbances can affect the condition of the vegetation and be observed at a sampling location. In addition to verifying the associated vegetation class, the field observer should document disturbances (Table SOP-1.5). This includes any recent management or natural disturbances that have changed the structure or composition of the vegetation ***over at least 10% of the sampling plot***. The disturbance should be detectable by the field observer at the time of the visit to the site; most observable disturbances will have occurred within the last two years. Some disturbances, such as tree blow-downs, may be visible much longer than two years and should be documented. If there is no observable change to the expected structure or composition of the vegetation (even if records indicate management took place; e.g. burning or grazing), then do not record as a disturbance.

1. Document up to 5 types of disturbances (Table SOP-1.5) observed at the survey location on the SOP 1 Data Sheet 1; record 'none' if no disturbances are observed.
2. Record the disturbance information during any repeat visits for collecting data.
    a. Site classifications do not need to be re-verified after the initial visit of a sampling year.

Frequency

Site location, set up, NVC verification, and the RAD are done one time only. Disturbances should be recorded on each **visit**.

Table SOP-1.5. Disturbances that may affect the structure and composition of the vegetation.

Disturbances	
Animal damage or disturbance	Invaded by exotic species
Chained	Mowed
Construction: building	Plowed/Disked
Construction: road	Prescribed burn
Construction: trail	Treated with fertilizer
Destructive use (non-harvest)	Treated with herbicide
Drought damage	Treated with insecticide
Flooded	Wetland: drained
Forest: clear-cut	Wetland: fall drawdown
Forest: selective harvest	Wetland: spring drawdown
Grazed	Wildfire
Hurricane damage	Wind event/blow down
Ice damage	Other (write in)
Insect damage	No disturbance

Marking Plots for Intensive Sampling

Once a RAD has been conducted at a sample of plot sites, those sites that provide a desired gradient of conditions for testing the SOPs 2, 3, and 4 will be determined. These plots will be sampled and measured on multiple occasions (every 3 weeks). To save time, transect start and end points should be established. Mark the three types of plots using Table SOP-1.6 as a guide and check list.

Table SOP-1.6. Additional marking of plots that will be sampled more intensively following examination of the RAD results.

		Placement within		
Marker	Purpose	Grassland (PRG, UPG, and CRP) Plots	Roadside (RDS) Plots	Agricultural fields with crops (AGC) Plots
Coordinates	Geo-referencing for relocating and spatially linking monitoring data	Four outer corners of the 300 x 75 m rectangular plot	Four outer corners (near start locations for Transects 1 and 9; at end points for Transects 8 and 16); use for photo points	Four outer corners of the rectangular area containing all transects. Use these corners for photo points
White flag	Marking ends of plant and immature monarch sampling transects	Start and end-points on long-side of plot, spaced 20 m apart (16 flags each along two, 300-m lengths)	Opposing corners of each diagonal transect; 10 meters apart; 8 on both sides of road (see Figure SOP-1.1c; 16 flags each on two, 670-m long roadsides)	Four corners of rectangle formed by beginning and end of first and last sequence of transects in crop rows; 5 m apart (32 flags/plot)
Pink flag; bamboo pole	Plot location and boundaries	Mid-points are 150 m from long-side end points--can be elevated on bamboo poles (6 each/plot)	Optional: 4 outer corners of plot demarcated by end of transects 8 and 16; and width distance away from starting points of transects 1 and 9 (4 flags/plot; no bamboo)	Optional: 4 outer corners of plot demarcated by end of select transects– number determined by length of crop rows (4 flags/plot; no bamboo)
Orange flag	Segments for butterfly walk	Along the perimeter every 75 m but between pink corners and long-side mid-points see Figure SOP 1.3 (8 flags /plot)	Set at start and end of 10 75-m long Segments, each oriented parallel to roadside, 6 meters from edge of the road (20 flags/plot)	Field dependent. Ideally, two sets of five, 75-m long Segments, situated in two 400-m long crop rows, with the 2 rows 75 m apart.

References

Comer P, Faber-Langendoen D, Evans R, Gawler S, Josse C, Kottel G, Menard S, Pyne M, Reid M, Schulz K, Snow K, Teague J. 2003. Ecological systems of the United States: a working classification on U.S. terrestrial systems. NatureServe, Arlington, VA.

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